American Journal of Medical Genetics 62:164–168 (1996)

Variant Rhizomelic Chondrodysplasia Punctata (RCDP) With Normal Plasma Phytanic Acid: Clinico-Biochemical Delineation of a Subtype and Complementation Studies

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Rhizomelic chondrodysplasia calcificans punctata (RCDP) is an autosomal recessive peroxisomal disorder which affects phytanic acid oxidation and de novo biosynthesis of plasmalogens in liver and fibroblasts. Peroxisomal thiolase is present in its unprocessed precursor form (44 kDa). We studied a mentally retarded 9-year-old girl with cataracts and atypical bone dysplasia. Neurological findings were mild compared to classic RCDP. Plasma phytanic acid was normal. Results of de novo plasmalogen synthesis and phytanic acid oxidation studied in cultured skin fibroblasts were intermediate between normal controls and classic RCDP. Peroxisomal thiolase was present only as the unprocessed 44 kDa protein. Taken together these results suggest that we are dealing with a variant form of RCDP with clinical and biochemical abnormalities much milder as compared to classic RCDP. In order to establish the genetic relationship between our patient and classic RCDP patients complementation studies were carried out. Earlier studies had already shown that fibroblasts from all RCDP patients studied belong to a single complementation group. Fibroblasts from our patient could also be assigned to this complementation group suggesting that the phenotypic variability results from different mutations within the same gene.

KEY WORDS: rhizomelic chondrodysplasia punctata, peroxisomal disorder, complementation, phytanic acid

INTRODUCTION

Autosomal-recessive rhizomelic chondrodysplasia punctata (RCDP) (McKusick 21500) is an inherited disorder of peroxisomal metabolism. It has a clinically distinct phenotype represented by dysplasia and shortening of long bones, periarticular calcification (calcific stippling), and cataracts. Affected patients usually present with severely stunted growth, are bedridden, and suffer severe mental deficiency and quadriplegia. The disease is often fatal early in life, but patients with prolonged survival are known [Spranger et al., 1971; Wardinsky et al., 1991]. Biochemical findings include impairments of phytanic acid oxidation, de novo biosynthesis of plasmalogens [Heymans et al., 1985, 1986; Hoefler et al., 1988; Schutgens et al., 1989, 1993], and the impaired posttranslational processing of peroxisomal thiolase [Heikoop et al., 1990], although the latter defect does not result in elevated very long chain fatty acids.

Recently patients have been described with biochemical findings similar to classic RCDP but with milder clinical expression, including atypical bone dysplasia without rhizomelic shortening [Pike et al., 1990; Poll-Thé et al., 1991; Smeitink et al., 1992; Nuoffer et al., 1994]. In these patients plasma phytanic acid was increased. We present another case of atypical RCDP, different from classical RCDP by its milder clinical and biochemical course. Furthermore the case is different from other atypical cases described by the finding of normal plasma phytanic acid. The patient is a 9-year-old daughter from nonconsanguineous Caucasian (Dutch) parents. She has a healthy 3-year-old brother. She appeared unremarkable at term birth (birthweight, 3,110 g) and was considered normal until 3 months when her mother noticed that her legs could not be straightened. Flexion contractures of the hips, elbows, and knees were found at 1 year. Bilateral cataracts necessitated lens extraction at 2

Received for publication April 25, 1995; revision received September 15, 1995.

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years. She sat unsupported at 2.5 years and walked independently at 8 years. She had no signs of spasticity, and had adequate hand control. She was able to express telegram style sentences. Speech development and adaptive behaviour at 8 years approximately represented a mental age between 1 and 2 years. Statural growth was −4 S.D. (partially due to flexion contractures of the hips) and head growth −3 S.D. at 15 months. She had somewhat unusual facial appearance, particularly an indistinct philtrum and a prominent nasal tip (Figs. 1, 2). Her appearance suggested coxa vara and femoral shortness (Fig. 3). X-ray studies at 5 years showed irregular growth plates of the vertebral column, ovoid vertebral bodies in the lumbar region and metaphyseal irregularities of long bones with some widening and fraying. Hip abnormalities consisted of bilateral coxa vara, and small femoral heads with delayed fusion (Fig. 4). X-ray findings of the knees at the age of 10 months showed irregular calcific stippling outlining the patellae (Fig. 5). This stippling had disappeared on repeat examination at 5 years. The length of the humeri and femora was measured between the epiphyses and compared to age controls. Tubular bone length measured between the epiphyses was at the 40th centile for the humerus, whereas the femoral length was at the 10th centile at the age of 5 years. This confirmed the impression of relative shortness of the femora, not explained by the dysplasia of the hips. Her EEG was normal at 1 year, as were somatosensory and brainstem auditory evoked responses. An MRI of the brain at 5 years showed normal myelination and no signs of dysplasia or atrophy. Standard metabolic screening tests including quantification of amino acids, organic acids, purines and pyrimidines, oligosaccharides, and acid mucopolysaccharides in the patient's urine gave normal results.

Fig. 3. Posterior aspect at age 9 years, with abnormality due to coxa vara and femoral shortness.

Figs. 1, 2. Facial appearance of patient at age 9 years. Slightly unusual appearance with indistinct philtrum and prominent nose tip.

MATERIALS AND METHODS

Standard methods were used for quantitative determination of very long chain fatty acids, bile acids, phytanic acid, pristanic acid, quantification of de novo plasmalogen synthesis, dihydroxyacetonephosphate acyltransferase (DHAP-AT), phytanic acid oxidation, and the identification of isoforms of peroxisomal thiolase [Schrakamp et al., 1989; Schutgens et al., 1989]. Complementation studies were performed in co-cultured fibroblasts from patients with classic RCDP and the present patient making use of [1-^{14}C]phytanic acid oxidation as a marker for complementation [Wanders et al., 1993]. Human skin fibroblasts were cultured in 75 cm^2

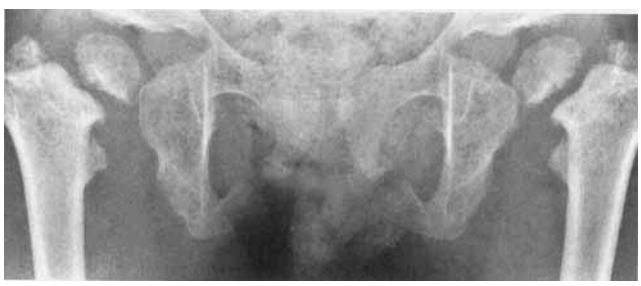

Fig. 4. X-ray view of pelvic skeleton at 5 years, demonstrating small femoral heads with delayed fusion.

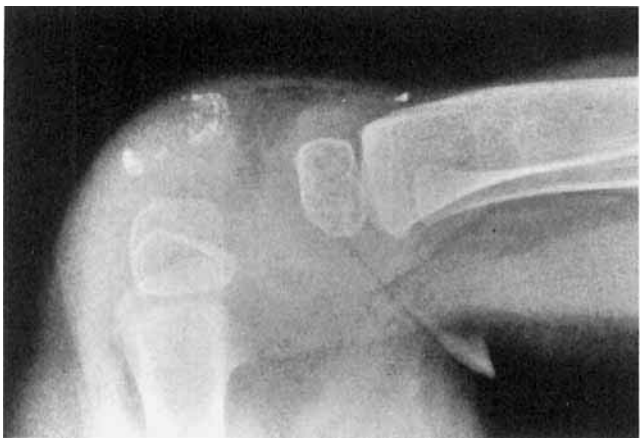

Fig. 5. X-ray view of patella at 10 months. Punctate calcification.

falcons in Dulbecco's Modified Eagle's Medium (DMEM) plus 10% fetal calf serum (FCS) and antibiotics. When the cells had reached confluence, they were harvested by trypsinization and 1.5×10^6 cells of each fusion partner were mixed and allowed to grow overnight. The next day the medium was removed and the cells were washed with DMEM. Subsequent fusion of cells was done as described [Brul et al., 1988]. The fused cells were grown overnight and harvested the next day by trypsinization. The procedure described by Nelson and Carey [1985] was used to separate mononucleated from multinucleated cells. Two fractions corresponding to 5% and 12.5% (w/v) Ficoll were collected separately, washed with DMEM fortified with HEPES (20 mM, pH 7.4), and the cells allowed to adhere overnight in DMEM supplemented with 2% (v/v) fetal calf serum. The following day the medium was replaced by DMEM without calf serum. After 72 h the phytanic acid oxidation capacity of the mononucleated and multinucleated cells was measured essentially as described [Wanders and van Roermund, 1993].

RESULTS

The results of pertinent peroxisomal function studies are given in Table I and the results of complementation in Table II.

Peroxisomal function parameters were essentially similar to classic RCDP, with normal very long chain fatty acids in plasma and in fibroblasts, absence of abnormal bile acids in plasma, decreased plasmalogens in plasma, impairment of de novo biosynthesis of plasmalogens in fibroblasts, decrease of DHAP-AT activity in platelets and fibroblasts, and the presence of the 46 kDa precursor of peroxisomal thiolase. Phytanic acid oxidation in fibroblasts was decreased, but residual capacity was above the range found in classic RCDP while plasma phytanic acid was not increased.

DISCUSSION

All patients with classic RCDP exhibit subnormal phytanic acid oxidation, leading to increased plasma phytanic acid, impairment of plasmalogen synthesis and the presence of unprocessed peroxisomal thiolase [Heymans et al., 1985, 1986; Hoefler et al., 1988; Schutgens et al., 1988]. The biochemical findings in the pre

TABLE I. Summary of the Biochemical Findings in the Patient, in Classic RCDP and in Controls*

	Patient	RCDP	Controls
Plasma			
Phytanic acid (μmol/L)	3.5	203.9 ± 174.1 (13)[a, b]	4.5 ± 1.6 (29)
Pristanic acid (μmol/L)	ND	0.1 ± 0.1 (6)	0.4 ± 0.4 (29)
Platelets			
DHAP-AT activity (nmoles/30 min. mg)	1.3	0.8 ± 0.5 (9)	3.5 ± 0.9 (136)
Erythrocytes			
Plasmalogens■			
($C_{16:0}$ DMA/$C_{16:0}$) × 100%	2.4	0.4 ± 0.4 (5)	8.8 ± 1.3 (30)
($C_{18:0}$ DMA/$C_{18:0}$) × 100%	5.6	0.3 ± 0.6 (5)	18.1 ± 3.4 (30)
Fibroblasts			
Phytanic acid oxidase activity (pmoles/48 h/mg protein)	2.60	1.9 ± 0.08 (6)	30.4 ± 5.3 (10)
Plasmalogens■			
($C_{16:0}$ DMA/$C_{16:0}$) × 100%	2.3	4.0 ± 0.8 (16)	12.1 ± 1.2 (24)
($C_{18:0}$ DMA/$C_{18:0}$) × 100%	1.0	1.7 ± 1.0 (16)	14.6 ± 2.8 (24)
DHAP-AT activity (nmoles/2 hr/mg protein)	4.3	1.9 ± 0.9 (39)	7.8 ± 2.0 (59)
Plasmalogen synthesis [$^3H/^{14}C$] ratio in		Mean (0–100% range)	Mean (0–100% range)
alkenyl PE	15.8	144 (42–400) (13)	0.7 (0.4–1.5)
alkenyl PC	6.5	7.4 (3.5–10.73)	0.6 (0.3–1.0)
Thiolase (Mr) (kDa)	44	44	41

*■DMA, dimethylacetals. ND, below detection level.
[a] Control values are given as their $\bar{x}$ ± s.d. except when indicated otherwise. Number of patients between brackets.
[b] Age at sampling above 12 months of age.

TABLE II. Complementation Studies*

RCDP1 × RCDP2	0.8
RCDP1 × Zellweger	4.1
RCDP1 × patient	0.9

*RCDP1 and RCDP2 represent fibroblast lines from patients with classic RCDP. Results are expressed as the ratio [1-^{14}C]phytanic acid oxidation in fused fibroblasts/[1-^{14}C]phytanic acid oxidation in co-cultured (unfused) fibroblasts.

sent patient are essentially similar to RCDP, but quantitatively different (Table I). Patients with the classic phenotype of RCDP are severely handicapped with profound mental impairment, spastic pareses, and cataracts. Typical metaphyseal bone dysplasia with shortness of the long bones, especially humeri and femora and severe contractures are present [Spranger et al., 1971]. Calcific stippling as visible on roentgenograms of the skeleton is present, but disappears after the age of 2 years. A review of old X-ray pictures showed patellar calcification at 10 months (Fig. 5). Typical shortness of tubular bones was not present in the humeri, but shortness of the femora, independent of the coxa vara deformity, could be demonstrated. Therefore, we conclude that the clinical findings essentially resemble those of classic RCDP. She also differs from the clinical phenotype with respect to mental and motor performance: she has no signs of spasticity and is able to walk (at 8 years) and communicate. While early studies [Spranger et al., 1971] suggested an early lethal course of RCDP, survival beyond 1 year appears not rare in later studies [Wardinsky et al., 1990]. Recently mild RCDP-variants have been described [Pike et al., 1990; Poll-Thé et al., 1991; Smeitink et al., 1992; Nuoffer et al., 1994]. The clinical course of these patients was mild compared to classic RCDP, while biochemical findings were essentially similar to classic RCDP. In these atypical RCDP patients phytanic acid was elevated between 5 [Nuoffer et al., 1994] and 197 times [Pike et al., 1990], whereas it was normal in our patient. Table I shows that the three parameters (phytanic acid oxidation, plasmalogen synthesis, and processing of thiolase) are all abnormal in our patient. Residual capacity for phytanic acid oxidation is intermediate between controls and classic RCDP, and this may explain the normal plasma phytanic acid level. In RCDP the block in phytanic acid oxidation is probably proximal to pristanoyl CoA oxidase leading to normal or only minimally increased pristanic acid concentration. In our patient pristanic acid was not elevated. Plasmalogens in the patient's erythrocytes were decreased and plasmalogen synthesis was impaired, as evaluated by in vitro synthesis, and by enzyme assay (Table I). But residual activities found were again intermediate between classic RCDP and normal controls. No complementation was found between a fibroblast line carrying classic RCDP and fibroblasts from the present patient (Table II). This finding indicates that the gene defect in our patient is at the same gene locus associated with classic RCDP [Heikoop et al., 1992]. The reason for the atypical expression is unexplained. An allelic mutation which results in higher residual enzymatic activities than classic RCDP could be involved. The normal phytanic acid in our patient suggests that clinical and biochemical expression in this atypical RCDP is mainly, if not exclusively due to the defect in plasmalogen synthesis. Recently patients have been described with isolated defects of peroxisomal enzymes subserving de novo plasmalogen synthesis. Isolated deficiency of alkyl dihydroxyacetone-phosphate acyltransferase was described by Wanders et al. [1992] and by Barr et al. [1993]. Isolated deficiency of alkyl dihydroxyacetone phosphate synthase was described by Wanders et al. [1994]. These patients typically displayed the clinical findings of classic RCDP. A recently described patient with isolated deficiency of dihydroxyacetone-phosphate acyltransferase had a milder phenotype [Clayton et al., 1994]. These cases of isolated plasmalogen synthesis defects also indicate that the defect in plasmalogen synthesis is the main factor involved in the clinical expression of RCDP. RCDP is the only chondrodysplasia which has been definitely classified as a peroxisomal disorder. Genetic peroxisomal disorders can be divided into three broad groups. Group A includes disorders of peroxisome biogenesis, such as Zellweger syndrome, with absent peroxisomes on routine electronmicroscopic staining and multiple peroxisomal pathways impaired. Group B includes multiple peroxisomal enzyme deficiencies with peroxisomes structurally intact, RCDP being the main representative of this group. Group C includes single peroxisomal enzyme deficiencies. Peroxisomal enzyme proteins are synthesized on free ribosomes and addressed to the peroxisome by topogenic signalling sequences on the respective proteins. Two main sequences consist of a tripeptide on the carboxyterminal, known as PTS-1, and an aminoterminal signal known as PTS-2. Each of them is essential for the import of a distinct series of peroxisomal proteins. In RCDP targeting by carboxyterminal tripeptides is normal, but the import of (peroxisomal) thiolase into the peroxisome is defective [Motley et al., 1994]. Because thiolase requires an aminoterminal label for its entry, RCDP may well be a deficiency of the putative PTS-2 receptor. A recent review [Purdue and Lazarow, 1994] summarizes the body of evidence which relates to mechanisms of peroxisomal protein import.

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